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Some observations upon the test for choline in human blood. (Abstract.) By R. W. Allen and Herbert French. (Communicated by M. S. Pembrey.)

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Professor Halliburton and Dr Mott have published extensive researches on the chemistry of nerve degeneration in the Croonian Lectures of 1900 and 1901, and in the *Philosophical Transactions of the Royal Society* 1899 and 1901, and have devised a test for choline in the blood. The details of this test will be found in an article by Dr Mott in the *Archives of Neurology*, vol. II. p. 859. The principle of it is to extract the blood four times in succession with absolute alcohol, to obtain the choline free from potassium, to precipitate the choline as choline platinochloride, and to crystallise the latter from 15% alcoholic solution. The crystals are yellow octahedra, and are readily seen under the low power of the microscope.

In the original description of the test the choline platinochloride was washed by decantation in absolute alcohol, before redissolving in 15% spirit and crystallising; a slight modification of the method of washing has since been introduced, namely to evaporate the absolute alcohol from the precipitated choline platinochloride at 40° C., to wash the dried residue with absolute alcohol, to redissolve the choline platinochloride in 15% spirit, and to allow the latter to crystallise spontaneously. This process, exact details of which will be given in a subsequent paper, has been employed throughout the present series of experiments; and the quantity of blood used has been 60 minims, or about 4 c.c.

Dr Mott states that the yellow octahedral crystals are obtainable from 5 c.c., or less, of blood from persons in whom active nerve tissue destruction is taking place; whereas "using 10 c.c. of normal human blood the results are practically negative, although frequently a few small octahedra may be found on careful examination." Wishing to investigate the value of this test from the clinical point of view, we succeeded in obtaining yellow octahedral crystals from a series of patients suffering from various nerve disorders. On the other hand, we also succeeded in getting similar crystals from 4 c.c. of our own blood, and from that of two apparently healthy students. This did not agree

with Dr Mott's statement, so we proceeded to investigate the test further. Pure choline carried through Mott and Halliburton's process gave the crystals readily, but there may be other substances in the blood which do the same. The platinochlorides of the following inorganic constituents of normal blood are yellow octahedra-K, Na, NH₃, Ca, Mg, Fe. We took the chloride of each separately and carried it through the process. The solubilities in absolute alcohol of the double platinochlorides are such that those of Fe, Mg, Ca, and Na, did not remain in the final residue, but we found yellow octahedra with both K and NH₃. Therefore we conclude that, although choline may be present in the blood of patients suffering from nerve disorders, this is not proved by obtaining octahedral crystals from their blood; such crystals, in Mott and Halliburton's process, may be the double platinochloride of at least three different substances, namely potassium, ammonium, or choline. Swale Vincent has recently stated that he believes them to be in great part the ammonio-platinochloride. Some may be; some may be due to the choline salt; but the following experiments seem to prove that many of the crystals are due to neither of these. We took blood, both normal and from patients with nerve disorders, and carried it through the process as far as the fourth extraction with absolute alcohol. We incinerated the dried extract in a porcelain crucible, using the blowpipe; and then continued the process. Yellow octahedral crystals were still obtained, possibly fewer than before, but still plentiful. Blowpipe incineration must have destroyed the choline and ammonium, so that these crystals were, we suggest, those of potassium platinochloride.

Without some modification, therefore, of Mott and Halliburton's process such as will ensure the previous elimination of both the ammonium and the potassium, these yellow octahedra do not prove the presence of choline in the blood. That choline may be present is possible, but the process does not prove it, and therefore it lacks value as a clinical test. We bring these results before the Physiological Society in order that their discussion may suggest further experiments, and possibly some modification of the test which may give it an important place in clinical medicine.

